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Applicants: David Henry Solomon et al.

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For: POLYMER GELS AND METHODS FOR THEIR PREPARATION

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Frank M. Gasparo, Esq. (Reg. No. 44,700) August 13, 2002
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SUBMISSION OF PRIORITY DOCUMENT

SIR:

Applicants hereby submit a certified copy of the priority document: Australian Provisional Application No. PR 2180 filed on December 19, 2000 in the name of the University of Melbourne.

The Commissioner for Patents is hereby authorized to charge payment of all fees associated with this communication to Deposit Account No. 02-0393.

Respectfully submitted,

Date: August 13, 2002

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I, JONNE YABSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PR 2180 for a patent by THE UNIVERSITY OF MELBOURNE filed on 19 December 2000.

I further certify that the above application is now proceeding in the name of GRADIPORE LIMITED pursuant to the provisions of Section 113 of the Patents Act 1990.

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Thirteenth day of December 2001

J R Yabsley

JONNE YABSLEY
TEAM LEADER EXAMINATION
SUPPORT AND SALES

AUSTRALIA

Patents Act 1990

~~The University of Melbourne~~
Gradipore Limited

PROVISIONAL SPECIFICATION

Invention Title:

Polymer gels and their preparation

The invention is described in the following statement:

Polymer gels and methods for their preparation

FIELD OF THE INVENTION

The present invention relates to three-dimensional gels, methods for their preparation and articles made or formed from these three-dimensional gels.

BACKGROUND

Three-dimensional aqueous gels (hydrogels) are covalently crosslinked hydrophilic polymers that are insoluble in water [1]. However, these gel networks establish equilibrium with the liquid and temperature of their surroundings for shape and mechanical strength [2]. Variations in the concentration, structure and/or functionality of the monomer and/or crosslinker used in such gels can change the gel structure, and this is reflected for example in the porosity of the network [3].

Conventionally, crosslinked polymer structures are produced by using a crosslinking agent in which the double bond has approximately the same as, or as close as possible to, the reactivity of the monomer used to form the linear part of the polymer. For example, a crosslinked polystyrene polymer is usually formed by the monomer styrene and the crosslinker divinylbenzene (DVB), where the reactivity of the double bond of DVB is approximately the same as styrene [4].

Generally the reactivity ratio (r) of two different monomers is defined as the reactivity of the radical from the first monomer reacting with the first monomer over the reactivity of the radical reacting with the second monomer:

Reactivity Ratio $r_1 = K_{11}/K_{12}$

Similarly,

Reactivity Ratio $r_2 = K_{22}/K_{21}$

Here K_{11} is the reaction rate of the radical from the first monomer reacting with the first monomer and K_{12} is the radical from the first monomer reacting with the second monomer.

In other words, the conventional approach used to form a crosslinked gel is by choosing similar reactivity ratio r_1 and r_2. When $r_1 = r_2 = 1$, during the network formation, the crosslinker enters the polymer chain in a statistical manner depending on the concentration. For an ideal system, if there is 1 crosslinker for every 10 monomers, the polymer network incorporates a crosslinker unit for every 10 units of the monomer.

Recently we reported crosslinkers that contain two slightly different reactive functional groups [5,6]. The functional group reactivity was a combination of two of

the following groups, acrylamide, methacrylamide, acrylate and methacrylate. The resultant gels were found to have enhanced protein separation in electrophoresis that has been attributed to the reactivity differences between the monomers.

These crosslinkers were used in an effort to control the network by delaying the reaction of one of double bonds by selecting a crosslinker in which one of the double bonds has the same reactivity as the monomer forming the linear part of the chain and the other is less reactive. These crosslinkers contain only two double bonds, and were designed to control the exotherm by delaying the reaction of the second double bond. This delayed reaction results in polymers that are less crosslinked in the earlier stage of the polymerization. Therefore the formed polymer with pending second double bond on its chain still has mobility and termination of the radical reaction continues and two chains can self annihilate (termination by combination or disproportionation), resulting in a controlled exotherm of the reaction. This occurs because in a free radical polymerization, a stage is reached where self-termination is prevented and this stage is influenced by the viscosity, and is called the gel-effect. At this point in the polymerization, self-termination is prevented, the chains cannot approach one another and the rate of monomer conversion is greatly increased with a consequently large exotherm.

We have made the surprising discovery that by using a crosslinker that has at least two double bonds with a greater reactivity than the monomer used to form the linear polymer, a polymer network (or gel) with unexpected but useful properties results. For example, a crosslinked polymer gel with an exceptionally high concentration of monomer and crosslinker (high T% and C%) was formed where the optical clarity of the gel is still relatively high. In addition the same crosslinked system can result in a polymer network with larger pores and enhanced sieving properties during electrophoresis.

We propose that these gel properties arise because the double bond of the crosslinking agent is more reactive than the double bond of the monomer, and enters the polymer chain more readily than the monomer/s resulting in a new pathway of polymer network formation. We believe this new pathway is controlled by the reactivity of the crosslinker, which influences the manner in which the network forms, by controlling the composition of the initially formed polymer. The manner in which the network forms is evident by the exotherm generated when two crosslinkers, with similar and variable double bond reactivity are compared (Figure 1). For example N,N-methylenebisacrylamide (BIS) has acrylamide type reactivity and the similarly shaped crosslinker N,N-methylenebismethacrylamide (mBIS) has the more reactive

methacrylamide type double bonds compared to acrylamide. From the existing gelation theory we expect the crosslinker with methacrylamide type reactivity such as mBIS to generate a relative large exotherm very quickly. However, the opposite was observed and the methacrylamide type crosslinkers such as mBIS produced a depressed exotherm during the free radical polymerization with the acrylamide monomer. This has lead us to believe that a heterogenous micro-phase structure is formed during the polymerization when the reactivity of the crosslinker is greater than that of the monomer/s. The microphase structure, which can also be called a star type structure, contains better chain mobility throughout the polymerization period and behaves essentially like a linear polymer, and does not give the expected exotherm.

Accordingly, in a first aspect, the present invention provides a crosslinked polymer system formed from at least one monomer and at least one crosslinker having a plurality of functional groups, wherein said functional groups has a greater reactivity than the monomer with one double bond.

Preferably the reactivity ratio (r) of the at least one crosslinker to the at least one monomer is in the range $r_1 = 0.001\text{-}0.8$; $r_2 = 1\text{-}6$. Preferably $r_1 = 0.05\text{-}0.1$; $r_2 = 1.3\text{-}4$.

Preferably the %T and %C of the polymer system is in the range of about 5%T, 3%C to 40%T, 15%C. These values are largely dependant on the specific application.

Preferably the polymer system of the first aspect of the invention is that of a hydrogel.

In a second aspect, the present invention provides a crosslinked polymer (hydrogel), which having a hetero microphase structure.

By the term "hetero microphase structure" is meant a gel network that is characterised by a plurality of highly crosslinked loci or cores interconnected by relatively linear polymer chains.

The functional groups of the crosslinker(s) used may be the same or different, where at least two or more of the functional groups are more reactive than the double bonds of the acrylamide monomer. The crosslinker may be a linear, branched or cyclic compound. Preferably all functional groups of the crosslinker have an ethylenic double bond. Particularly preferred crosslinkers are those described in Applicant's International Application No. PCT/AU00/00238, the disclosure of which is incorporated herein by reference.

The crosslinkers used may be the mixture of two or more types of crosslinkers, including the conventionally used crosslinker with the same reactivity. The mixed system may be used to provide both properties of the traditional gel structure and new

polymer network in accordance with the present invention. For the maximum effect, the double bond with the lowest reactivity from the crosslinker/s should be higher than the highest reactivity of the monomer/s.

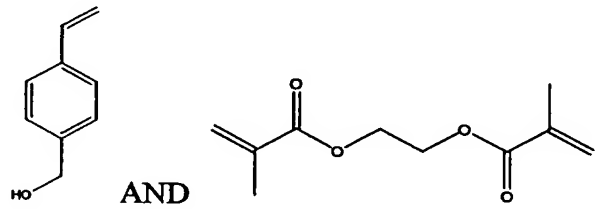
The monomer or monomers used may be any suitable monomer. The gel may be formed from two or more different monomers.

The polymer system may be prepared from one or more monomers having the formula $H_2C=CR_5\text{-}CO\text{-}N(R_3)R_4$ where R_3, R_4 are each independently H, alkyl, alcohol ($\text{-}(CH_2)_n\text{-}OH$), or ester ($\text{-}(CH_2)_n\text{-}OCH_3$), where n is 1-6, and R_5 is H or optionally substituted alkyl. Examples of monomers include acrylamide, acrylamide derivatives or acrylamide substitutes known to the art such as N,N-dimethylacrylamide, methacrylamide, N-methyloylacrylamide, propylacrylamide, dipropyl acrylamide, isopropyl acrylamide, diisopropyl acrylamide, lactyl acrylamide, methoxyacrylamide and mixtures thereof.

The polymer system may be formed from a monomer system of acrylamide (AAm) with methylenebismethylacrylamide (mBIS) or other crosslinker which has greater reactivity than AAm, such as 2-hydroxyethyl methacrylate.

The polymer system can be formed from non-acrylamide type monomers such as ester type systems. Example of such system would be hydroxyethyl acrylate (HEA) as the monomer with the more reactive ethyleneglycol dimethacrylate (EGDMA) as the crosslinker or combine with other crosslinkers. Non-limiting examples of other suitable monomer/crosslinker are shown as follows:

a)



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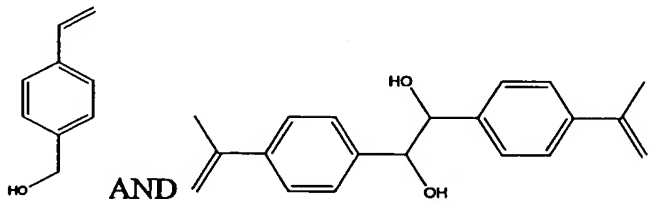
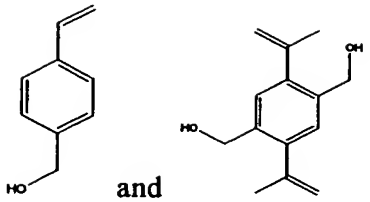


c)



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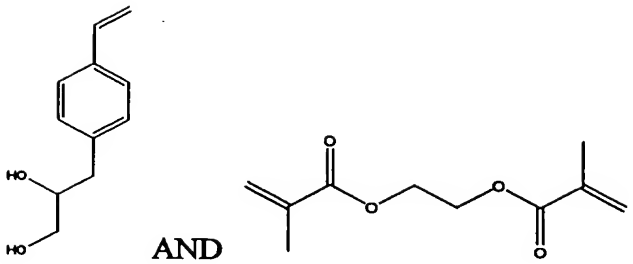
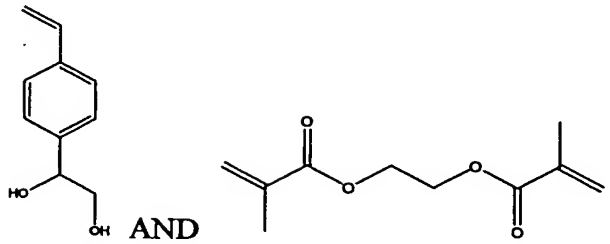
d)



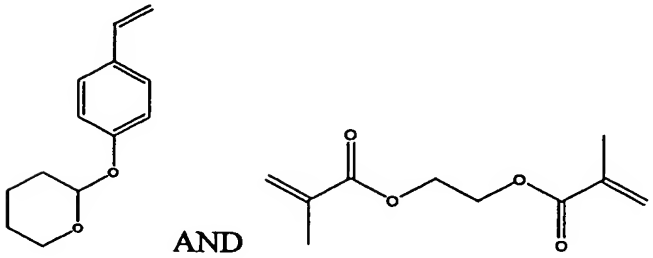
AND

e)



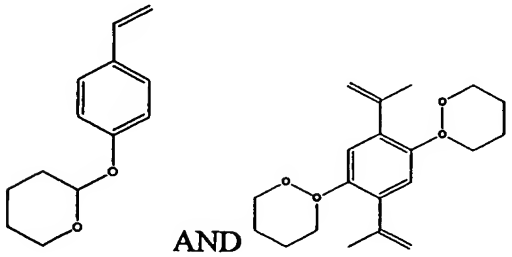
AND

f)



AND

g)



AND

Particularly preferred are those monomers used to produce hydrogel intraocular lenses and biological separation matrices and the like.

Due to the nature of this new polymer system of the present, it is possible to produce higher crosslinked gels with high optical clarity that is not producible with conventional methods. This property is particular useful in the lens industry where manufacturing harder and clear lens is desirable.

On the other hand, by applying this new technology, it is also possible to produce a polymer network with large pore sizes that cannot be obtained using the conventional method due to the low concentration of the crosslinking points. This is particular useful in membrane applications for the separation of large bio-molecules in electrophoresis.

Accordingly, in a third aspect, the present invention provides an article formed at least in part from a polymer gel in accordance with the first of second aspects of the present invention.

The article may be an optical lens, for example, a contact lens.

The article may be an electrophoresis gel, which may or may not have a porosity gradient, composition gradient or concentration gradient. The gradient may be achieved by using different concentrations of the polymer gel or by altering the ratio of crosslinker to monomer.

The electrophoresis gel may have a porosity gradient suitable for gradient gel electrophoresis. See for example, *Polyacrylamide Gel Electrophoresis across a Molecular Sieve Gradient* Margolis, J., Kenrick, K.G., Nature, 214, 1967, p1334-1336; *Polyacrylamide Gel Electrophoresis in a Continuous Molecular Sieve Gradient,* Margolis, J., Kenrick, K.G., Analytical biochemistry, 25, 1968, p347-362; and *Practical System for Polyacrylamide Gradient Gel electrophoresis,* Margolis, J., Laboratory Practice, 22, p107-109, 1973, the disclosures of which are incorporated herein by reference.

The polymer gel of the present invention may be in the form of a membrane.

Accordingly, in a fourth aspect the present invention provides an electrophoretic gel formed from a polymer system in accordance with the invention.

In a fifth aspect, the present invention provides an electrophoretic membrane including an electrophoretic gel in accordance with the present invention formed on a porous substrate.

The substrate supply the support frame for the electrophoretic medium. The substrate may be a porous paper or fabric. The substrate may be woven or non-woven sheet, for example, a non-woven PET.

The greater control on designing gels with a different pore size range and/or distribution provided by the polymer gels of the present invention make them particularly suitable for use in electrophoresis separation method and apparatus described in Gradipore Limited's US Patent No. 5,039,386 and US Patent No. 5,650,055, the disclosures of which are incorporated herein in their entirety. This technology is incorporated into Gradipore Limited's Gradiflow™ technology. The technology allows for the separation of macromolecules such as proteins, nucleotides and complex sugars. It can be used for size separation, concentration and dialysis. A commercially available form of this technology is Gradipore Limited's Gradiflow™ BF200 unit. The heart of Gradiflow™ is a membrane cartridge, which consists of three polyacrylamide-based membranes. The top and bottom membranes are small pore size restriction membranes. These membranes allow the movement of small ions, The middle membrane is the separating membrane, which varies with the particular application. This middle membrane usually has a larger, but defined pore size. It is in this middle membrane that the membrane of the present invention may have particular application. For specific applications, the membrane may be charged or have an affinity ligand embedded within the membrane.

By applying mixed monomers containing charged group, together with the crosslinker with great reactivity, it can produce a polymer network with porosity controlled by external stimuli.

The above described pore size of the polymer network with dialable porosity can be controlled by for example the pH of the solution or the voltage applied on the polymer during its electrophoresis.

By applying mixed monomers containing part of the monomer with specific conformation, together with the crosslinker with great reactivity, it can produce a polymer network with diallable porosity by changing this specific conformation.

The above described pore size of the polymer network with diallable porosity can be controlled by using specific wavelength under photolysis to switch the conformation in one way or the other.

In a sixth aspect the present invention provides a method for forming a crosslinked polymer gel system in accordance with the invention, the method including reacting at least one crosslinker with at least one monomer, wherein the at least one crosslinker has a greater reactivity than the at least one monomer.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

In order that the present invention may be more readily understood, we provide the following non-limiting embodiments of the invention.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1. is a schematic diagram showing the molecular structure of crosslinkers used to make polyacrylamide gels and membranes described below;

Figure 2. shows the temperature profile over time during the free radical polymerization of AAm with different crosslinkers;

Figure 3. shows the amount of water swelling changes at 120 min with the change of T% of a polyacrylamide gel crosslinked by either BIS or mBIS under constant 3 C%.

Figure 4. shows the amount of water swelling changes at 120 min with the change of T% of a polyacrylamide gel crosslinked by either BIS or mBIS under constant 7 C%;

Figure 5a & 5b. is a Ferguson plot and the migration patterns obtained for the polyacrylamide gels containing different crosslinkers after fractionation by PAGE of a broad range protein standard;

Figure 5c & 5d. shows the Rf difference for a broad range protein standard after PAGE with polyacrylamide gels containing multifunctional crosslinkers compared to the BIS crosslinked polyacrylamide gels;

Figure 6. The difference of Rf value obtained from protein electrophoresis between the gel crosslinked with mBIS and BIS under the formulation of 15T%/3C%.

Figure 7. The difference of Rf value obtained from protein electrophoresis between the gel crosslinked with mBIS and BIS under the formulation of 30T%/3C%.

Figure 8. The difference of Rf value obtained from protein electrophoresis between the gel crosslinked with mBIS and BIS under the formulation of 15T%/7C% and 5T%/7C%.

Figure 9. The difference of Rf value obtained from protein electrophoresis between the gel crosslinked with mBIS and BIS under the formulation of 10T%/5C%.

Figure 10. The difference of Rf value obtained from protein electrophoresis between the gel crosslinked with mBIS and BIS under the formulation of 20T%/5C%.

Figure 11. shows SEM images obtained for 10%T 3%C polyacrylamide gels crosslinked with BIS, 1a and 1b.

Figure 12 Clarity Comparisons between HEMA/EGDMA and HEA/EGDMA Gels

EMBODIMENTS OF INVENTION

Abbreviations

Acrylamide (AAm), N,N'-methylenebisacrylamide (BIS), polyacrylamide gel electrophoresis (PAGE), Scanning electron microscopy (SEM), N,N'-methylenebismethacrylamide (mBIS), hydroxyl ethyl acrylate (HEA), ethylene glycol diacrylate (EGDA), hydroxyl ethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA).

General Procedure for preparing Polyacrylamide gels

Example 1: Synthesis of the crosslinkers

1,3,5-triacrylylperhydro-*s*-triazine (1a) [7] and 1,3,5-trimethacrylylperhydro-*s*-triazine (1b) [8] were synthesized as previously reported. The monomers Bis and mBis were of electrophoresis grade and used without further purification. The monomers were recrystallized from alcohol and employed after purification was confirmed. Monomers 2a-5b were prepared, as we have recently reported [9].

Example 2: Preparation of the stock monomer solution

A 30%T 3%C stock solution is made up by dissolving acrylamide (29.10g) with the selected crosslinker either Bis (0.90g), mBis (1.06g), 1a (0.97g), 1b (1.13g), 2a (1.20), 2b (1.36), 3a (1.03g), 3b (1.20g), 4a (1.06g), 4b (1.22g), 5a (1.28g), or 5b (1.45g) in a 100ml volumetric flask with distilled water. The solution is filtered through a Whatman No. 1 filter paper and stored at 4°C prior to use. A 40%T 10%C stock solution was similarly made with AAm (36.0g) and the selected crosslinker Bis (4.0g), mBis (4.73g) in a 100ml volumetric flask with distilled water. Dissolving AAm (40g) in a 100ml volumetric flask made a 40%T 0%C stock solution.

Example 3: Preparation of the polyacrylamide gels

For all crosslinking agents with different potential functionality (different number of polymerizable groups), substitutions were calculated on a mole basis (not on a weight basis) with Bis. When the potential functionality varied between the crosslinkers, the substitutions were calculated on an equivalent number of double bond basis with Bis. For an AAm and Bis system the accepted terminology of %T refers to the total concentration of the monomer AAm and the crosslinker Bis as a percentage (w/v). The term %C refers to the concentration of the crosslinker Bis (w/w) as a portion of %T. The crosslinkers used with a potential functionality greater than four were calculated on an equivalent basis where the number of double bonds initially in the solution are the same. That is, for every 1 mole of Bis, 2/3 of a mole of a hexafunctional crosslinker is required. This formulation will result in the real value of %T and %C of each PAAm gel crosslinked with a crosslinker other than Bis to vary from the Bis crosslinked AAm system. For example a 10%T 3%C gel would contain 9.7g of AAm and 0.3g of Bis per 100ml. An equivalent 10%T 3%C solution containing the hexafunctional crosslinker 1a would require 9.7g of AAm and 0.32g of the crosslinker 1a. This results in an actual concentration of 10.02%T 3.19%C for the AAm and 1a system. For simplicity, the concentrations used refer to Bis crosslinked gels and all the other crosslinked systems with similar concentrations are referred to as the equivalent Bis %T and %C concentration.

A polyacrylamide gel solution (10ml) was prepared by mixing the required amounts of the appropriate stock monomer solution (3.33ml), distilled water (4.17ml) and 1.5 M Tris-HCl buffer (pH 8.8) (2.5ml). The 1.5M Tris-HCl buffer was made by dissolving Tris (27.23g) in water (80ml), adjusting the pH to 8.8 with 6N HCl, and diluting to 150ml with distilled water. The polyacrylamide gel solution was degassed by vacuum aspiration at room temperature for 40 minutes and then purged with nitrogen until the initiator system was added. The initiator system was composed of freshly made up 10% (w/v) APS (0.025ml) and 10% (v/v) TEMED (0.025ml) where the mole ratio of APS to TEMED was kept constant at 1:1. The gel solution (7ml) was immediately cast between two glass cassettes (8 x 8cm, 1mm apart) purging with nitrogen and left to polymerize for at least 3 hours.

Example 4: Conversion of monomer and crosslinker into polyacrylamide gels

The degree of copolymerization between AAm and a crosslinker towards a three-dimensional polymer network was measured using a HPLC system. The polyacrylamide gel made as above was removed from the glass cassette, weighed, crushed in a beaker and washed with methanol three times to extract the unreacted residual acrylamide and crosslinker. The methanol washing's were combined, filtered and made up to 50ml in a volumetric flask. A 50µl sample of this solution was injected into the HPLC with methanol as the elutant and with a detecting wavelength at 254nm. This wavelength was chosen because all unreacted double bonds are detected and it reaches a good compromise between sensitivity and convenience. The peaks observed for the unchanged monomers were measured against an acrylamide standard curve to calculate the concentration and amount of unreacted double bonds.

General procedure for analysis of polyacrylamide gels with different crosslinkers compared to Bis

Example 5: Polymerization temperature profiles

The monomer and crosslinker solution (4ml) was prepared as described above and cast into a small glass vial purged with nitrogen. The vial containing a thin temperature probe began to record the temperature as soon as the monomer solution was poured into the glass vials, initiated and the glass vials were capped. The temperature probe readings were taken every 30 seconds for 2 hours and a temperature profile of the polymerization reaction was obtained.

Example 6: Water swelling

The amount of water absorbed and the degree of swelling of a polyacrylamide gel was measured. A piece of the polyacrylamide gel (5 x 5cm) as made above was cut, weighed and dried in a 60°C oven for 24 hours. The dry gel was then weighed and immersed in 100ml of distilled water, at 20°C. Every 10 minutes for 2 hours the gel was removed from the water, patted with filter paper to remove any excess surface water, weighed and returned to the water.

Example7: Polyacrylamide gel electrophoresis (PAGE)

SDS-PAGE were performed and prepared under the discontinuous conditions of Laemmli [10]. A stacking gel with a concentration of 5%T 3%C was similarly prepared to the gels made above. The stacking gel (1-2ml) was poured into the top of the glass cassettes already containing the resolving polyacrylamide gel made above to make the mould for the protein wells required for electrophoresis. SDS-PAGE was performed with a constant voltage of 150V and 500mA for one hour using a constant power supply, a Gradipore micrograd vertical electrophoresis unit and a TRIS electrophoresis running buffer. The TRIS buffer was prepared by dissolving Tris (9g), SDS (3g) and glycine (43.2g) in 100ml of distilled water and diluting 1:5 with distilled water before use. A 10 µl broad range protein marker was microsyringed into the sample wells embedded in the stacking gel and separated using Electrophoresis. The gels were stained after electrophoresis with a Coomassie brilliant blue G250 stain for 24 hours and then destained with 10% acetic acid to visualize the protein migration pattern.

Example 8: Scanning Electron Microscopy (SEM)

A piece of a polyacrylamide gel (5 x 5 cm) was made as above and mounted vertically on a SEM stub with a non-conductive glue and cryogenically fractured in liquid nitrogen. The water was sublimed at -95°C for 90 minutes and then the sample was cooled to -198°C, coated with platinum using argon gas and plasma for 2 minutes. The images of the fractured polymer were then taken at various magnifications.

<u>Gel characterization results of polyacrylamide gels</u>

Example 9: Novel multifunctional Crosslinkers

The structural design of the crosslinkers used for the formation of polyacrylamide gels is shown in Figure 1. A systematic investigation to correlate the reactivity of the

crosslinker with the properties of the polyacrylamide three-dimensional network was carried out. The double bonds of the crosslinkers were either of acrylamide type reactivity (Bis, 1a, 2a, 3a, 4a and 5a) or methacrylamide type reactivity (mBis, 1b, 2b, 3b, 4b and 5b) and all crosslinkers were soluble in aqueous AAm solutions. The relative monomer reactivity towards polyacrylamide radicals has been reported to be 1.00 for acrylamide and 1.35 for methacrylamide type double bonds [11].

Example 10: Polymerization characterization

Whilst not essential to invention we believe that it is helpful to speculate a reason behind all variations. However, this is not essential for the invention. Initially, the extent of the polymerization or the degree of the monomer and crosslinker double bonds reacted were measured to ensure a reproducible three-dimensional network was formed, and the observations made were representative of the true gel network. All the gels tested had greater than 99% monomer conversion which were considered satisfactory for further analysis and applicable for bio separations.

The network formation of a free radical polymerization is a kinetically controlled process where the addition reaction of the monomer double bonds during the chain growth is exothermic [12]. Measuring the temperature increase over time we can monitor this exothermic free radical polymerization and provide a measure for the amount of AAm incorporated into the polymer network over time.

The change in temperature and the polymerization rates between the monomer acrylamide and a crosslinker is shown in Table 1. The curve obtained contains a flat line (induction period) at the beginning of the reaction, which is sensitive to inhibitors such as oxygen, which may delay the onset of the polymerization. This is followed by a sharp rise in temperature. The gradient of this rise is used to calculate the rate of the polymerization and the maximum change in temperature. The polymerization rate was slower and the 'Trommsdorff' effect was slightly depressed for PAAm gels crosslinked with the methacrylamide type crosslinkers compared to the equivalently structured acrylamide type crosslinkers.

Table 1: Temperature range and polymerization rates during the free radical polymerization of acrylamide with different crosslinkers

Crosslinker	Temperature change	slope (°C/mins)
BIS	11.2	0.559
1a	10.6	0.499
2a	9.5	0.389
3a	11.1	0.540
4a	8.2	0.261
5a	6.2	0.260
mbis	9.0	0.278
1b	3.7	0.083
2b	6.9	0.166
3b	1.3	0.008
4b	1.7	0.008
5b	3.0	0.024

Example 11: Polyacrylamide gel optical clarity

At a concentration of 10%T 3%C 1a crosslinked gels were slightly cloudy upon the onset of the gel point despite being less reactive and more hydrophilic than 1b. Polyacrylamide gels crosslinked with 1b were clear at 10%T 3%C. This phase separation was attributed to the formation of a tightly packed network which exudes water from the three dimensional network. 1a presumably forms a tight and rigid network with AAm, which the water (solvent) cannot penetrate to push the chains apart, and solvate the gels. However, the gel crosslinked with 1b has equivalent functionality to 1a but is clear and transparent. This was also observed for the Bis and mBis system. Opaque gels were reported to form when the concentration was greater than 5%C for BIS crosslinked polyacrylamide gels [13]. Comparisons of optical clarity between BIS and mBIS were made and the results are shown in Table 2 and Table 3. At concentrations below 5%C both BIS and mBIS were transparent even at 40%T. At 10%T 5%C BIS gels started going cloudy and at 10%T 7%C BIS had become opaque. Whilst mBIS were slightly cloudy at 10%T 7%C and were not completely opaque until a concentration of 10%T 20%C was reached. The difference in the hydrophilic and hydrophobic balance between BIS and mBIS or 1a and 1b does not appear to be a determining factor in this system. This phenomenon was related to the different

pathways taken for the formation of the polymer network, which must be linked to the reactivity of the crosslinker.

The crosslinker mBis and 1b has more reactive methacrylamide double bonds than 1a, BIS and AAm. Generally, during the early stages of the polymerization the crosslinker mBis and 1b will be incorporated into the polymer chain much earlier than acrylamide resulting in loci of highly concentrated crosslinked areas. Once mBis or 1b is consumed into the polymer network, the remaining AAm in the solution will continue to react and build relatively linear polymer chains branching away from these crosslinked loci, linking them together and forming the resultant three-dimensional polymer network. During the elongation of the AAm polymer chains there is considerable flexibility and mobility within the reaction mixture to allow termination of the radicals present on the growing chains. Therefore, we observe a smaller 'Trommsdorff' effect with mBis and 1b compared to Bis and 1a respectively.

Table 2 Optical clarity of polyacrylamide gels crosslinked with BIS

		C								
	BIS gels	**0.5**	**1**	**2**	**3**	**5**	**7**	**10**	**15**	**20**
T	**2.5**				0					
	5				0	0	2			
	10	0	0	0	0	2	3	3	3	3
	40				0					

Table 3 Optical clarity of polyacrylamide gels crosslinked with mBIS

		C								
	mBIS gels	**0.5**	**1**	**2**	**3**	**5**	**7**	**10**	**15**	**20**
T	**2.5**									
	5						0			
	10					0	1	2	2	3
	15				0	1	2			
	20				0	1	3	3		

0 represents a clear gel
1 represents a slightly cloudy gel
2 represents a cloudy gel
3 represents an opaque gel

Surprisingly the gels crosslinked with mBIS and 1b were clear and remained transparent at relatively high concentrations and their polymerization rate was slower and the 'Trommsdorff' effect was depressed compared to BIS and 1b gels respectively. This phenomenon contains similar characteristics to that previously observed during the formation of novel crosslinked microgels in our lab [14,15]. We propose that mBIS and 1b have a core in which the crosslinker is concentrated and from which the relatively linear acrylamide arms grow. These particles appear to be approaching a microgel indicating a greater mobility of the chains compared to the case of BIS and 1a. That is, more self-termination is occurring. Microgels are 'intramolecularly' crosslinked macromolecules in solution of colloidal dimensions that are usually swollen and transparent [16]. The microgels synthesized in our lab are star shaped macromolecules that contain small highly crosslinked loci of crosslinked polymer particles that have lots of long chains connecting them together.-This type of polymer network results in-microgels being completely solvated and resulting in a transparent gel regardless of the concentration of monomers and crosslinkers used. It is envisaged in the polyacrylamide network with mBIS and 1b as the crosslinker. The quick and initial incorporation of mBIS and 1b into the polymer creates a number of highly crosslinked loci which are small due to the low concentration of mBIS and 1b used compared to AAm. Once mBIS and 1b was consumed into the polymer network, AAm begins to react and build polymer chains branching away from these crosslinker loci and linking them together forming a three-dimensional polymer network. During the elongation of the acrylamide polymer chains there is still considerable mobility within the reaction mixture. This allows termination of the radicals present on the chains to take place. Therefore, we do not observe a dramatic 'Trommsdorff' effect as seen in Figure 2 by the shape of the mBIS and 1b curve since the gel will be solvated in water similar to that observed for microgels.

Example 12: Water swelling properties

The content and degree of swelling of polyacrylamide gels in water was measured since water has such an important presence within the gel network. The amount of water absorbed by each gel was calculated as a ratio of water absorbed by the gel (g), divided by the dry gel (g) over time.

The water swelling test by varying C% under constant T at 10% are shown in Fig. 3, Fig. 4 respectively for both BIS and mBIS cross-linked polyacrylamide gels.

The swelling tests where C was kept at 3% (Fig.3), show that for both BIS and mBIS gels, the water uptake decreased as T was increased. This was a reflection of the physical properties of the gels. Gels of low T% are soft and flexible, allowing them to swell and take up water. Gels of high T% are harder and brittle, not allowing as much swelling, so the gels take up little water. The decrease in water uptake as T% was increased was more dramatic in the mBIS gels than the BIS gels. The water uptake of the mBIS gels was higher than the water uptake of the BIS gels of equivalent concentrations. The difference in water uptake between the BIS and mBIS gels became larger as T was decreased.

Similarly the swelling tests of the gels with C=7% (Fig. 4) show that as T was increased the water uptake of the gels decreased. For T=5% the mBIS gel had a significantly higher water uptake than the BIS gel. However, for values of T greater than 10% the BIS and mBIS gels had similar water uptake. At high concentrations the gels water swelling properties appears to be a reflection of the hydrophobic nature of the monomers.

Example 13: Electrophoresis (PAGE)

Electrophoresis is an established technique for separating biomaterials by size and/or net electrical surface charge density [17], where fractionation by size depends on the porosity of the gel network [18]. The pore size and pore size distribution of different crosslinked polyacrylamide gels was indirectly related to the crosslinker by investigating and comparing the electrophoretic migration pattern of protein standards by size along the gel using the electrophoresis techniques SDS- PAGE.

To correlate the crosslinker structural characteristics to the porosity of the gel, the Retardation factor (R_f) which is the distance migrated by each protein fraction divided by the distance traveled by the dye front was calculated. The Rf difference for each protein fraction separated on the new crosslinked gel compared to that of the standard BIS crosslinked gel was calculated and the results are shown in Figure 5c and 5d. Maintaining a constant gel concentration of 10%T 3%C, polyacrylamide gels crosslinked with methacrylamide type reactive crosslinkers which have greater protein separation than their respective acrylamide type crosslinkers

Example 13 Electrophoresis comparison of BIS and mBIS gels

In addition the difference between the R_f values of BIS and mBIS cross-linked polyacrylamide gels have been plotted for each of the protein bands that could be identified. A comparison between BIS and mBIS at a concentration of T=15% and C=3% is shown in Fig. 6 and 7. The protein bands appear to travel further through mBIS gels compared to BIS gels, except for the smaller proteins of log(MW)=4.491. The four bands between log(MW) 4.653 and 5.065 travel significantly further in mBIS than BIS. There appears to be little difference in the Rf values for the proteins of low molecular weight. For the T=15%, C=3% gels, the mBIS gel allows proteins (especially large proteins) to travel more easily through its gel network, suggesting there are larger pores in the gel structure. The water swelling tests back this up with the T=15%, C=3% mBIS gel having a much larger water uptake than the BIS gel, see Fig. 10.

A comparison between T=5%, C=7% and T=15%, C=7% BIS and mBIS gels is shown in Fig. 8. The three protein bands detected for each gel concentration have greater R_f values in the mBIS gel than the BIS gels. This shows that the 5/7 mBIS gels will have a network with larger pore sizes than the 5/7 BIS gel. The swelling tests support this with the 5/7 mBIS gel having a greater water uptake than the 5/7 BIS gel. The electrophoresis shows that the 15/7 mBIS gel has larger pore size than the 15/7 BIS gel. This is contradictory to the swelling tests, which showed the 15/7 BIS, and mBIS gels to have the same water uptake, see Fig. 11.

Electrophoresis was also performed on 10/5 and 20/5 BIS and mBIS gels, as can be seen in Figures 9 and 10. The comparison of the 10/5 gels shows that the protein bands in mBIS have higher R_f values than in the BIS gel. However, the comparison of the 20/5 gels show that the two gels have fairly similar R_f values, where the mBIS gel has allowed the larger proteins to migrate further than the BIS gel, but the smaller proteins have migrated further in the BIS gel than the mBIS gel. This can be explained by the pore size distribution of mBIS compared to the relatively uniform structure of BIS gels. The small proteins travel relatively easily through the pores of BIS but the areas of the mBIS structure that are highly cross-linked and have small pores, hinder

the migration of the proteins. The large proteins have difficulty moving through the BIS gel structure but the presence of areas with a looser matrix and little cross-linking in the mBIS structure allows for freer movement of these large proteins.

From these results it appears that the mBIS gels have a looser structure and slightly larger pores than the BIS gels. The greatest difference between the structures of the BIS and mBIS occurred at low T (T=5%). As T increased the difference between the structure of BIS and mBIS gels became less. When T reached 30%, there was very little difference between the pore structures of the gels cross-linked with BIS or mBIS.

Overall in this set of experiments, it was found that polyacrylamide gels cross-linked with mBIS have bigger pore sizes than polyacrylamide gels cross-linked with BIS with equivalent concentrations. The differences in pore sizes between mBIS and BIS gels of equivalent T% and C% concentrations, was found to be greatest when T% was small and the difference minimal when T% was larger. The pore size of the gel structure was found to decrease as either T% or C% increased. This was found to be true for polyacrylamide gels cross-linked with either BIS or mBIS.

Example 14: SEM observations

Polyacrylamide gels crosslinked with BIS have been studied using SEM [19-21]. It was shown by Ruchel and Brager [19] that a gel freeze dried at low temperatures could sublime the water from its pores whilst maintaining its structure without shrinking or introducing artifacts. However, a standard and very precise preparation method is needed for every gel compared, because the temperature and the time of water sublimation from the surface of the gel can alter the apparent pore sizes observed. The images taken of the gels crosslinked with BIS, mBis, 1a and 1b are shown in Figure 11 and clearly show a variation in the pore sizes and pore size distribution of each gel. The gels crosslinked with mBis and 1b appear to have a greater pore size distribution, where highly crosslinked areas have smaller pores surrounded by low crosslinked areas that have larger pores than Bis and 1a gels respectively.

Example 15 Preparation of Membrane From HEMA and EGDMA (36%T/3.6%C)

A 50 ml solution containing the monomer, hydroxyl ethyl methacrylate (HEMA) (17.3944g) and the crosslinker ethylene glycol dimethacrylate (EGDMA) (0.6428g) is degassed with argon until the oxygen level in the solution was below 3 %. The solution was then transferred to a membrane-making tower (size 190 × 80 ×100 mm) followed by the addition of the initiator (10%) APS (0.25 ml) and the co-initiator TEMED (0.12 ml). Five membranes was then cast between glass plates where a non-wove PET substrate (pre-treated with 10% BL18 surfactant) was used as a support. The reaction was allowed to polymerize for at least 3 hours before the membrane was taken out. The membranes were washed with distil water before used in Gradiflow® for protein separation.

Example 16 Preparation of Membrane From HEA and EGDMA (32.3%T/4%C)

A 50 ml mixture solution containing the monomer, hydroxyl ethyl acrylate (HEA) (15.5211) and the crosslinker, ethylene glycol dimethacrylate (EGDMA) (0.6429g) was degassed with argon until the oxygen level in the solution was below 3 %. The solution was then transferred to a membrane-making tower (size 190 × 80 ×100 mm) and initiated using the initiator (10%) APS (0.5 ml) and the co-initiator (10%)TEMED (0.24 ml). Five membranes were then cast between glass plates where a non-wove PET substrates (pre-treated with 10% BL18 surfactant) were used as support. The reaction was allowed for at least 3 hours before the membrane was taken out, washed with distil water and used in Gradiflow® for protein separation.

Example 17 Preparation of Membrane from HEA and EGDA (31.8%T/2.4%C)

A 50 ml solution containing the monomer, hydroxyl ethyl acrylate (HEA) (15.5211)and the crosslinker, ethylene glycol diacrylate (EGDA) (0.5519g) was

degassed with argon until the oxygen level in the solution was below 3 %. The solution was then transferred to a membrane-making tower (size 190 × 80 ×100 mm) and immediately initiated with the initiator (10%) APS (0.5 ml) and the co-initiator TEMED. (10%) (0.24 ml). Five membranes were then cast between glass plates where non-wove PET substrates (pre-treated with 10% BL18 surfactant) were used as support. The reaction was allowed for at least 3 hours before the membrane was taken out. The membranes were washed with distil water before used in Gradiflow® for protein separation.

Example 18 Clarity Comparison between HEMA/EGDMA and HEA/EGDMA Gels

Equivalent 50 ml monomer stock solutions, were prepared using the following formulations:

Stock solution 1 for HEMA/EGDMA gel:	17.3944g HEMA and 0.6428g EGDMA
Stock solution 1 For HEA/EGDMA gel:	15.5211 HEA and 0.6428g EGDMA

The stock solutions were diluted with water using the formulation below and degassed with Ar for 10 min. The gels were cast in Petri dishes (∅ 50 mm): under Ar blankets. Each of these samples were initiated with 0.2ml of 10%APS and 0.096 ml TEMED and the solutions were left to polymerize. The clarity was observed and recorded by scanning. Figure 8 shows the scanned gel, which demonstrate that under the same molarity (with equivalent amount double bonds), HEMA/EGDMA gels are opaque (sample 1 and 2) while HEA/EGDMA gels are more clear.

Sample 1:	5 ml	solution 1,	5ml water	(18T%/3.6%C)
Sample 2:	2.5 ml	solution 1,	7.5ml water	(9%/3.6%C)
Sample 3:	5 ml	solution 2,	5ml water	(16T%/4%C)
Sample 4:	2.5 ml	solution 2,	7.5ml water	(8T%/3.6%C)

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Claims

1. A crosslinked polymer system formed from at least one monomer and at least one crosslinker having a plurality of functional groups, wherein at least one of said functional groups has a greater reactivity than the at least one monomer.

2. A crosslinked polymer system according to claim 1 wherein the reactivity ratio (r) of the at least one crosslinker to the at least one monomer is in the range r_1 = 0.001-0.8; r_2 = 1-6. Preferably r_1 = 0.05-0.1; r_2 = 1.3-4.

3. A crosslinked polymer according to claim 1 or claim 1 wherein the %T and %C of the polymer system is in the range of about 5%T, 3%C to 40%T, 15%C.

4. A crosslinked polymer system according to any one of the preceding claims wherein the system is that of a hydrogel.

5. A crosslinked polymer system according to any one of the preceding claims wherein the functional groups of the crosslinker(s) are the same or different.

6. A crosslinked polymer system according to any one of the preceding claims wherein the at least one crosslinker has two functional groups.

7. A crosslinked polymer system according to any one of claims 1-5 wherein the at least one crosslinker at least three functional groups.

8. A crosslinked polymer system according to any one of the preceding claims wherein the at least one crosslinker includes one more other crosslinkers having substantially the same reactivity as the at least one monomer.

9. A polymer system according to any one of the preceding claims wherein the at least one monomer is selected from a compound having the formula $H_2C{=}CR_5\text{-}CO\text{-}NR_3R_4$ where R_3, R_4 are each independently H, alkyl, alcohol ($\text{-}(CH_2)_n\text{-}OH$), or ester ($\text{-}(CH_2)_n\text{-}OCH_3$), where n is 1-6, and R_5 is H or optionally substituted alkyl.

10. A polymer system according to any one of the preceding claims wherein the at least one monomer is selected acrylamide, acrylamide derivatives or acrylamide

substitutes known to the art such as N,N-dimethylacrylamide, methacrylamide, N-methyloylacrylamide, propylacrylamide, dipropyl acrylamide, isopropyl acrylamide, diisopropyl acrylamide, lactyl acrylamide, methoxyacrylamide and mixtures thereof.

11. A polymer system according to any one of claims 1-8 wherein the at least one monomer is selected from a compound having the formula R1-(R2)C=C(R3)-R4-(R6)C=C(R7)-R8. Usually, R1,R2, R3, R6, R7 and R8 are H or alkyl groups such as methylgroup. R4 is the linkage between two doubles.

12. An electrophoretic medium comprising a crosslinked polymer system according to any one of the preceding claims.

13. An electrophoretic medium according to claim 12 wherein the polymer system has a porosity gradient or a composition gradient.

14. An electrophoretic membrane, the membrane including a crosslinked polymer system according to any one of claims 1 to 10 formed on a porous substrate.

15. An intraocular lens comprising a crosslinked polymer system in accordance with any one of the preceding claims.

16. A crosslinked polymer hydrogel, the hydrogel having a hetero microphase structure.

17. A crosslinked polymer hydrogel according to claim 16 wherein the %T and %C of the hydrogel is in the range of about 5%T, 3%C to 40%T, 15%C.

18. A crosslinked polymer hydrogel according to claim 16 wherein the hydrogel is formed from at least one monomer selected from a compound having the formula $H_2C=CR_5\text{-}CO\text{-}NR_3R_4$ where R_3, R_4 are each independently H, alkyl, alcohol ($\text{-}(CH_2)_n\text{-}OH$), or ester ($\text{-}(CH_2)_n\text{-}OCH_3$), where n is 1-6, and R_5 is H or optionally substituted alkyl.

19. A crosslinked polymer hydrogel according to claim 18 wherein the at least one monomer is selected acrylamide, acrylamide derivatives or acrylamide substitutes known to the art such as N,N-dimethylacrylamide, methacrylamide, N-

methyloylacrylamide, propylacrylamide, dipropyl acrylamide, isopropyl acrylamide, diisopropyl acrylamide, lactyl acrylamide, methoxyacrylamide and mixtures thereof.

20. A crosslinked polymer hydrogel according to claim 16 wherein the hydrogel is formed from at least one monomer is selected from a compound having the formula R1-(R2)C=C(R3)-R4-(R6)C=C(R7)-R8. Usually, R1,R2, R3, R6, R7 and R8 are H or alkyl groups such as methyl group. R4 is the linkage between two doubles.

21. An electrophoretic medium comprising a crosslinked polymer hydrogel according to claim 16 .

22. An electrophoretic medium according to claim 21 wherein the hydrogel has a porosity gradient or a composition gradient.

23. An electrophoretic membrane, the membrane including a crosslinked polymer hydrogel according to claim 16 formed on a porous substrate.

24. An intraocular lens comprising a crosslinked polymer hydrogel according to claim 16.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia before the priority date of each claim of this application.

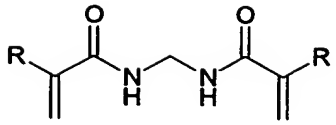
It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Dated this nineteenth day of December 2000

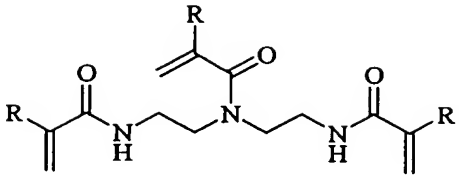
Gradipore Limited
~~The University of Melbourne~~

Patent Attorneys for the Applicant:

F B RICE & CO



R=H, bis
R=CH_3, mbis



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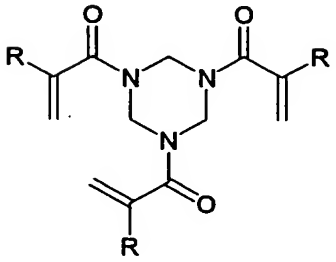
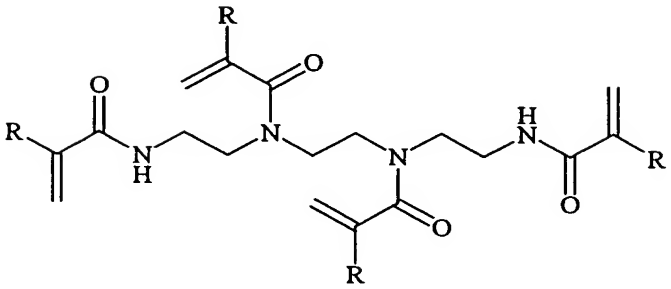
R=CH_3, 3b



R=H, 1a
R=CH_3, 1b



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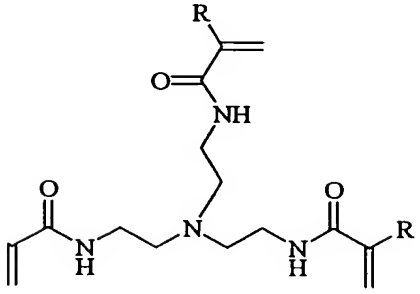

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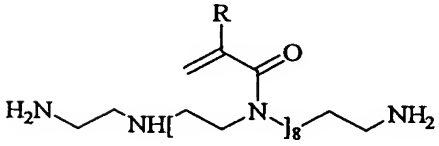


R=H, 5a
R=CH_3, 5b

Figure 1

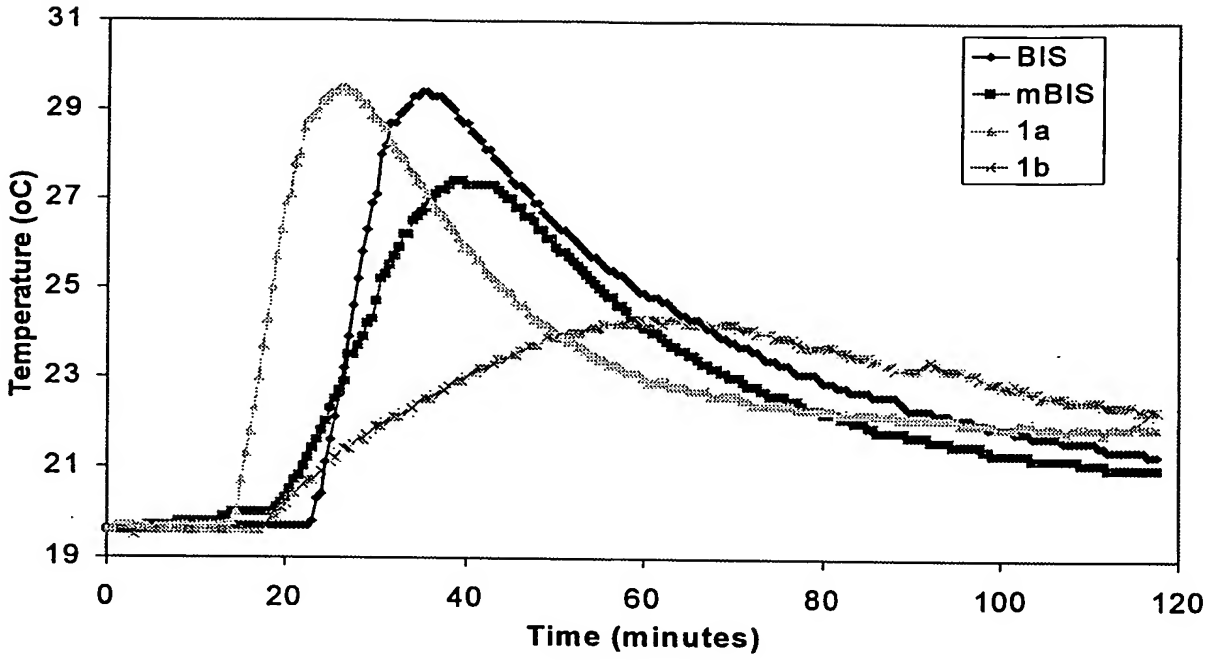

31
29
27
25
23
21
19
Temperature (oC)
0
20
40
60
80
100
120
Time (minutes)
BIS
mBIS
1a
1b

Figure 2

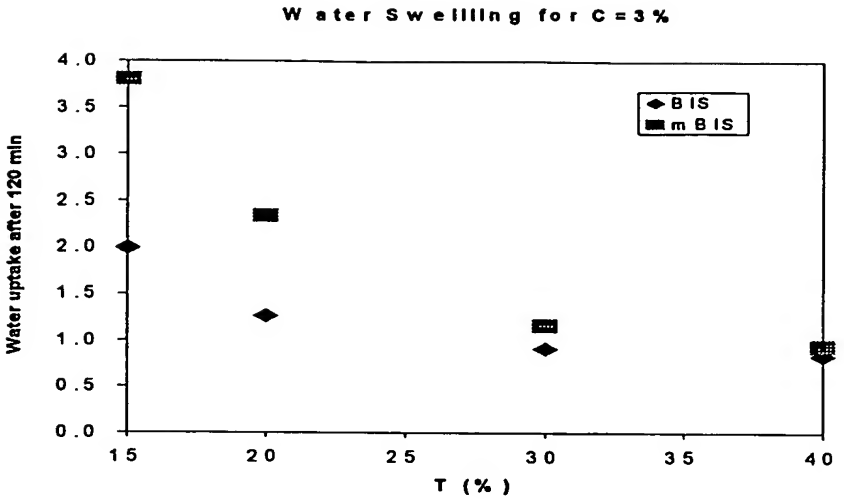

Water Swelling for C=3%
Water uptake after 120 min
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
15
20
25
30
35
40
T (%)
BIS
mBIS

Figure 3

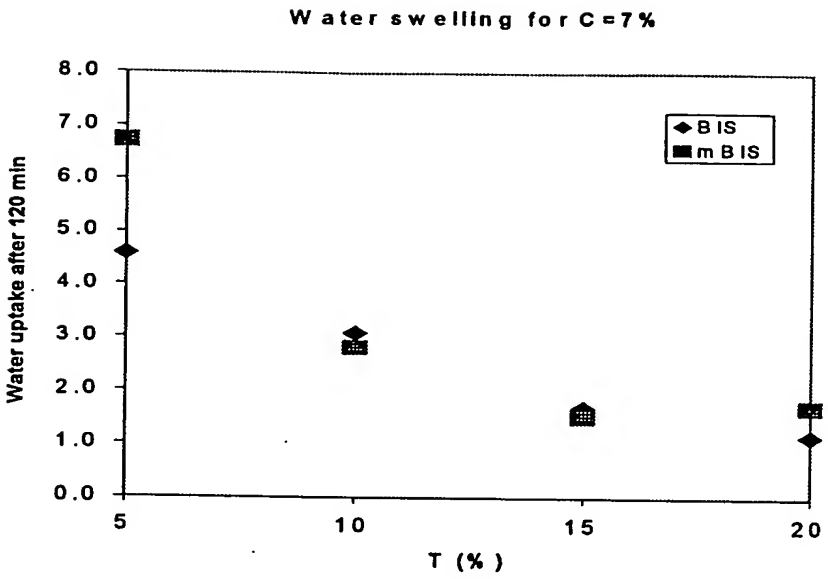

Water swelling for C=7%
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Water uptake after 120 min
5
10
15
20
T (%)
B IS
m B IS

Figure 4

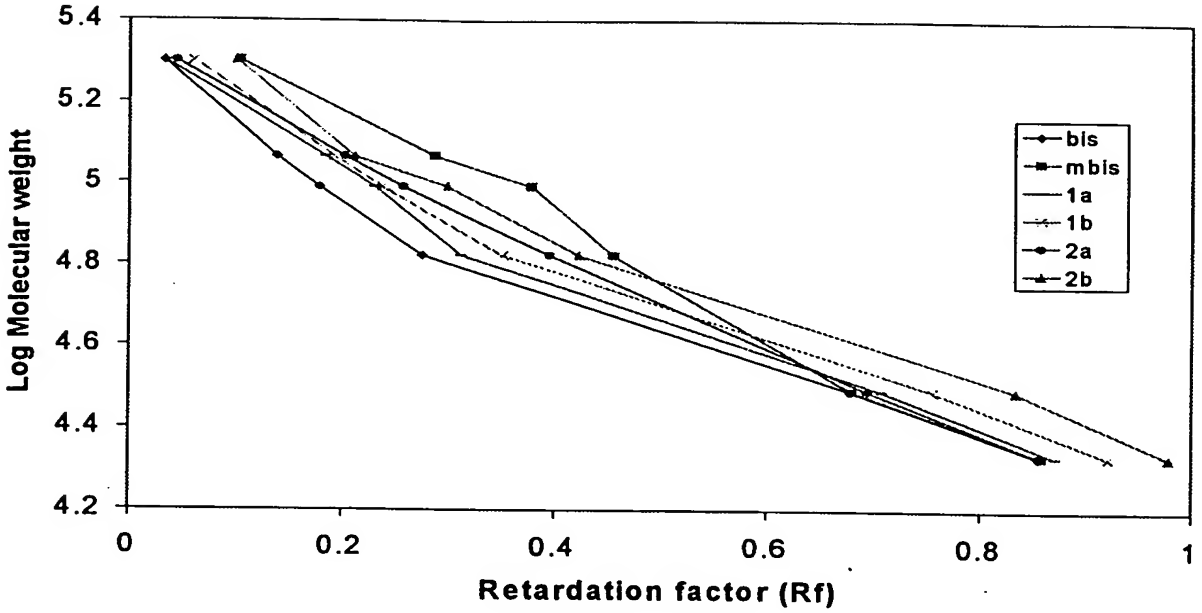

5.4
5.2
5
4.8
4.6
4.4
4.2
Log Molecular weight
0
0.2
0.4
0.6
0.8
1
Retardation factor (Rf)
bis
mbis
1a
1b
2a
2b

Figure 5a

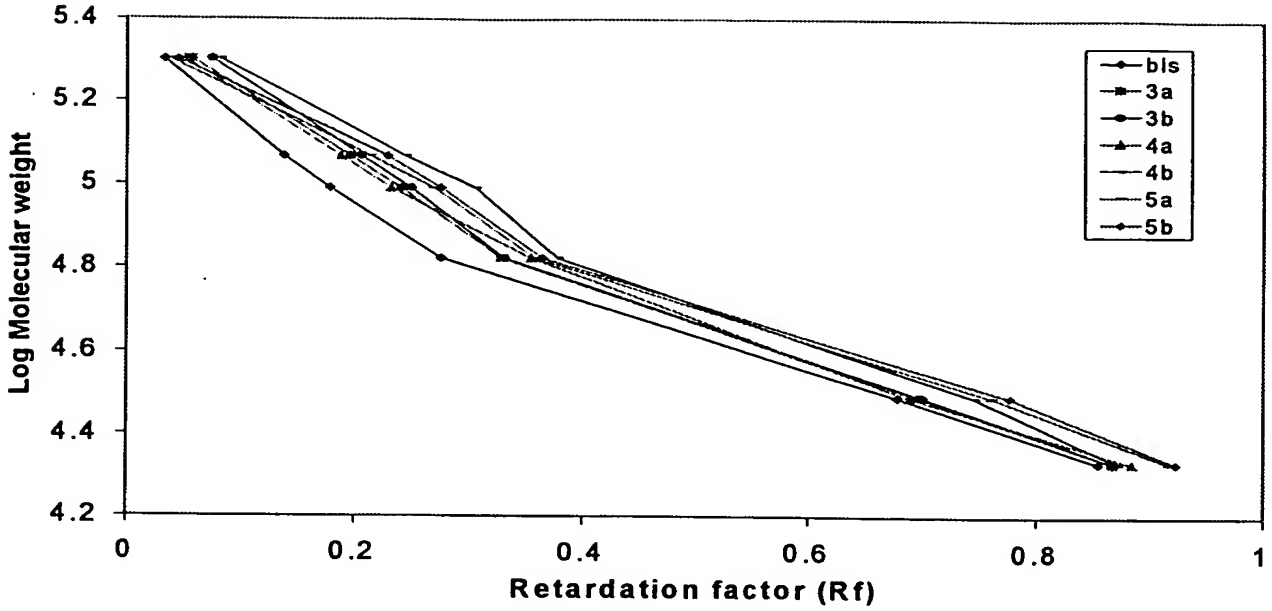

5.4
5.2
5
4.8
4.6
4.4
4.2
Log Molecular weight
0
0.2
0.4
0.6
0.8
1
Retardation factor (Rf)
bls
3a
3b
4a
4b
5a
5b

Figure 5b

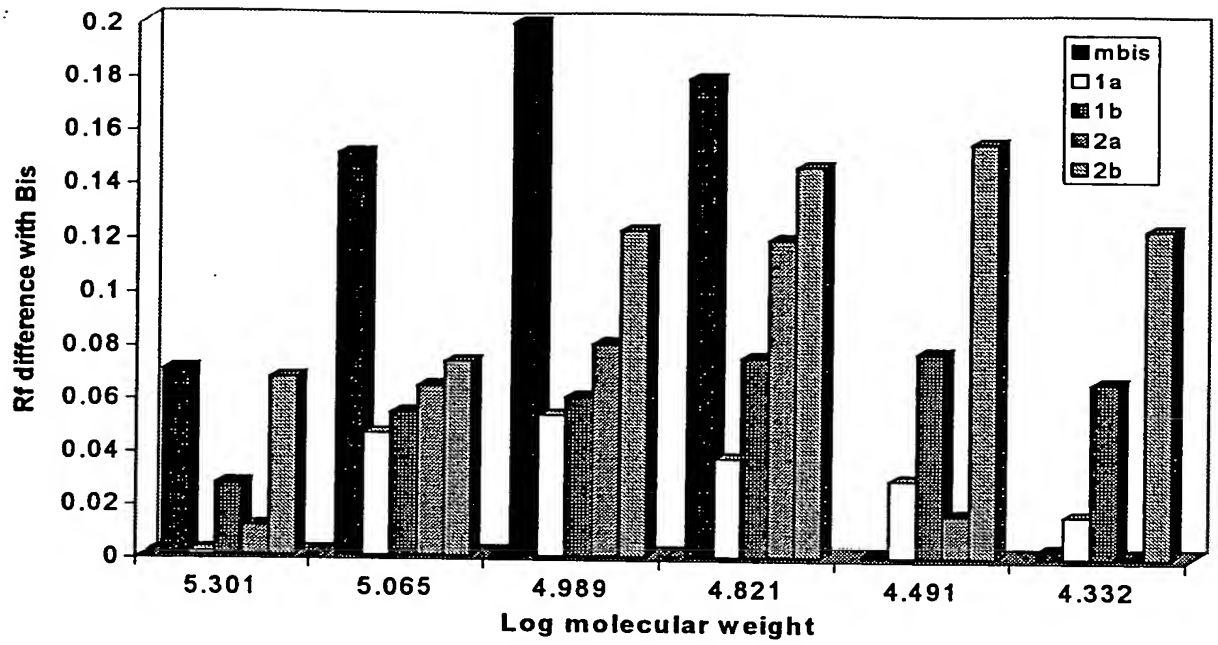

0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Rf difference with Bis
mbis
1a
1b
2a
2b
5.301
5.065
4.989
4.821
4.491
4.332
Log molecular weight

Figure 5c

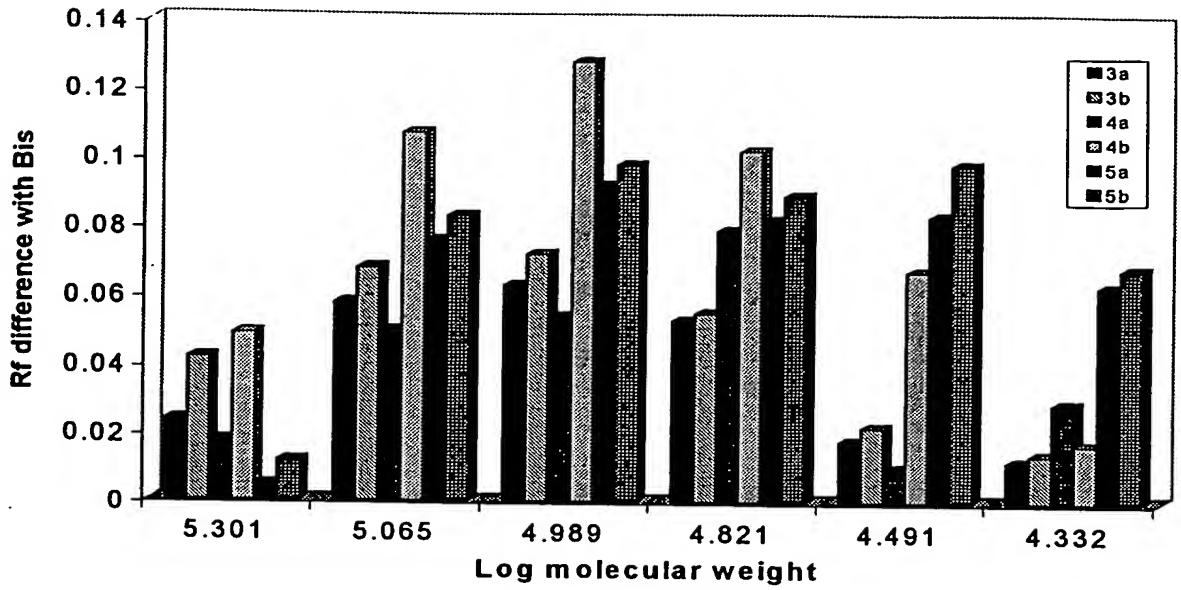

0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Rf difference with Bis
3a
3b
4a
4b
5a
5b
5.301
5.065
4.989
4.821
4.491
4.332
Log molecular weight

Figure 5d

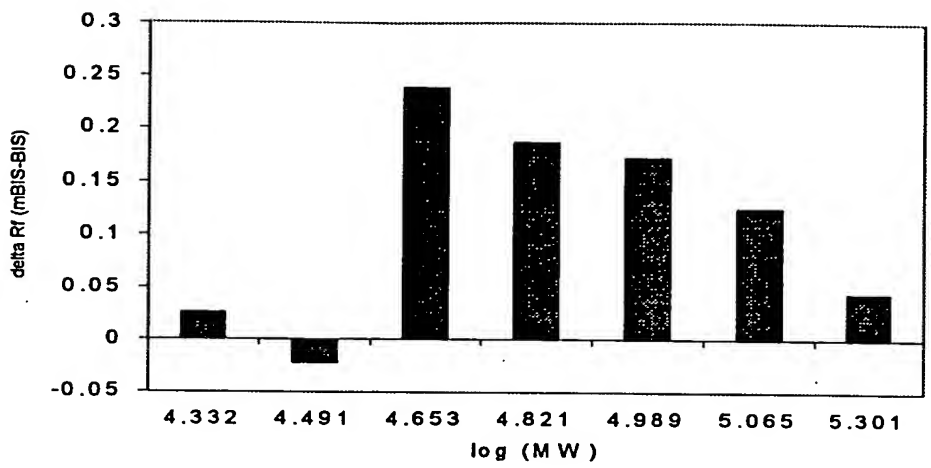

0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
delta Rf (mBIS-BIS)
4.332
4.491
4.653
4.821
4.989
5.065
5.301
log (MW)

Figure 6

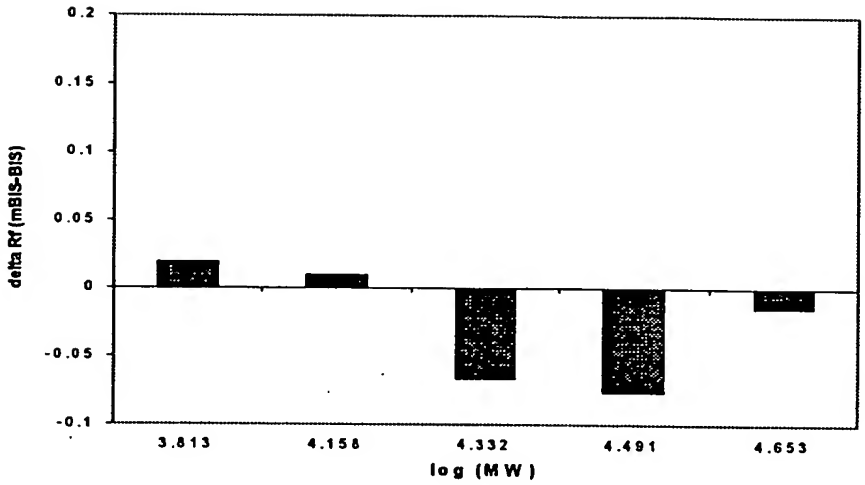

0.2
0.15
0.1
0.05
0
-0.05
-0.1
delta Rf (mBIS-BIS)
3.813
4.158
4.332
4.491
4.653
log (MW)

Figure 7

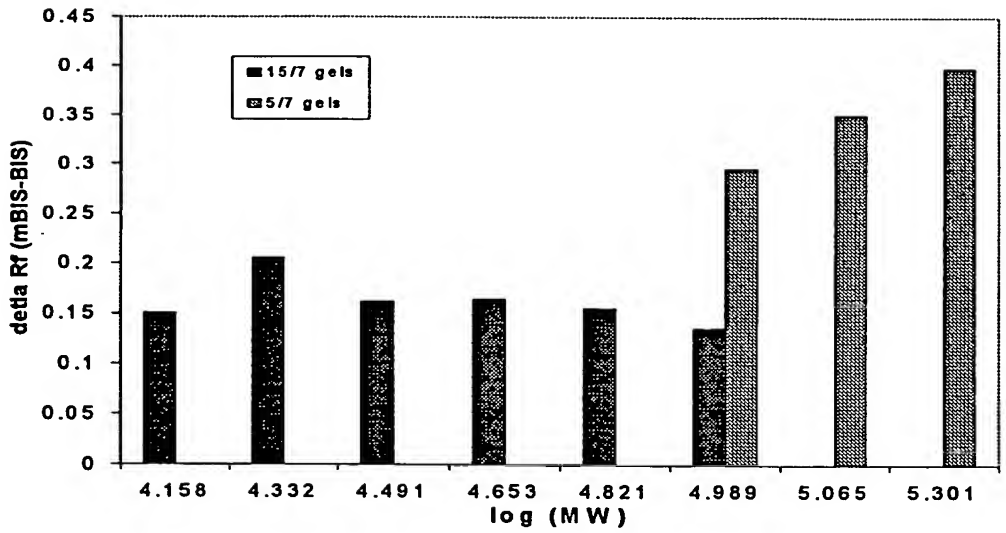

0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
delta Rf (mBIS-BIS)
15/7 gels
5/7 gels
4.158
4.332
4.491
4.653
4.821
4.989
5.065
5.301
log (MW)

Figure 8

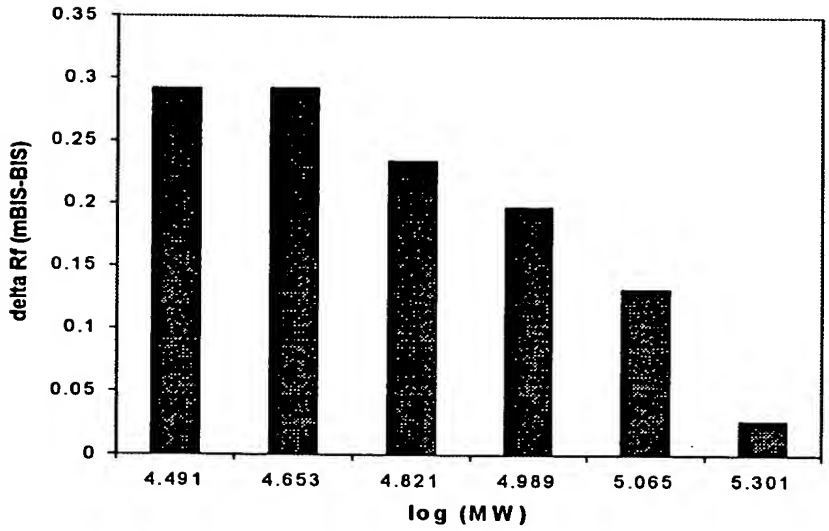

0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
delta Rf (mBIS-BIS)
4.491
4.653
4.821
4.989
5.065
5.301
log (MW)

Figure 9

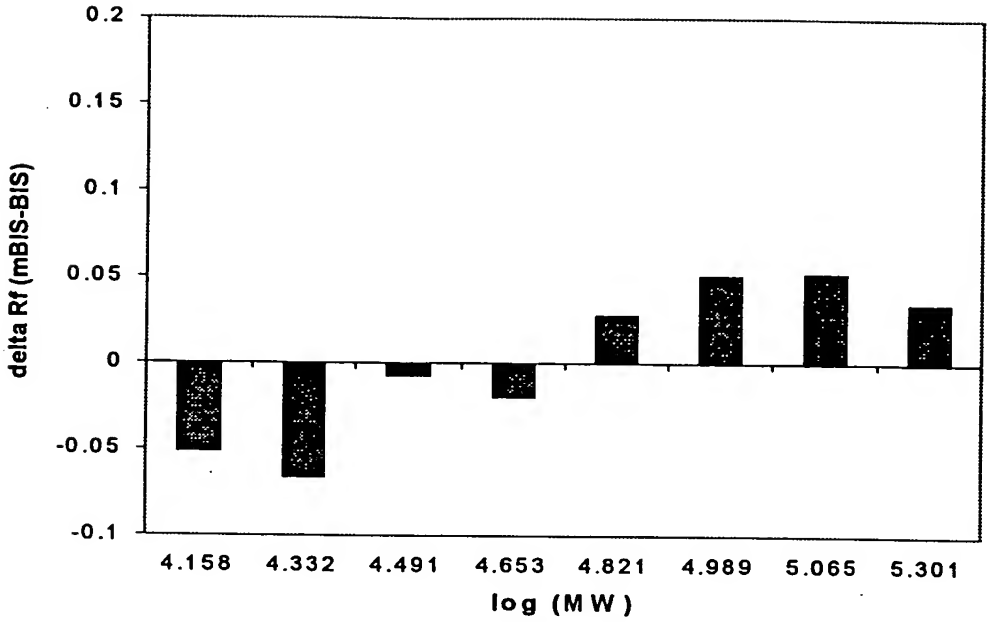
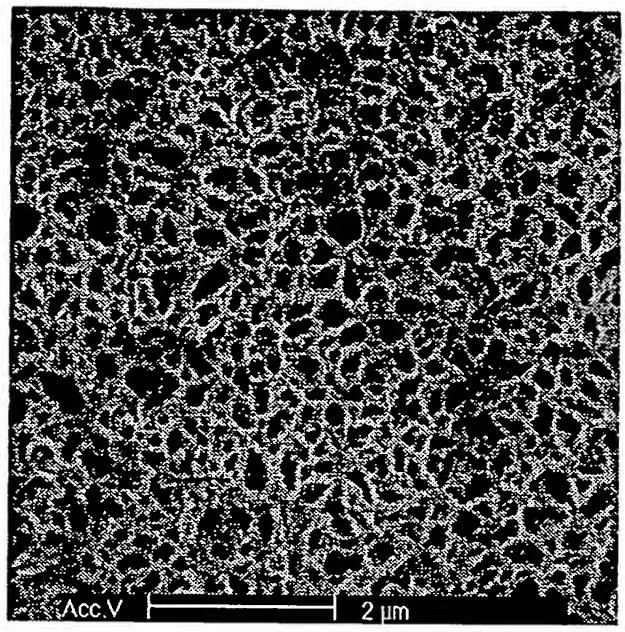
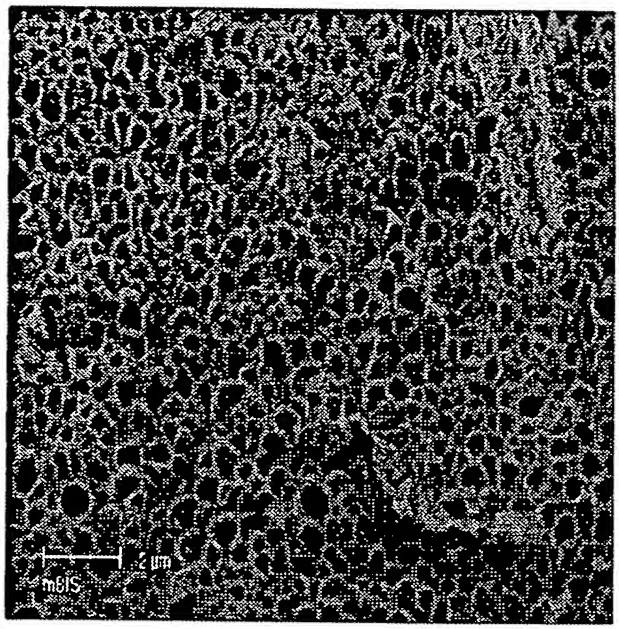

0.2
0.15
0.1
0.05
0
-0.05
-0.1
delta Rf (mBIS-BIS)
4.158
4.332
4.491
4.653
4.821
4.989
5.065
5.301
log (MW)

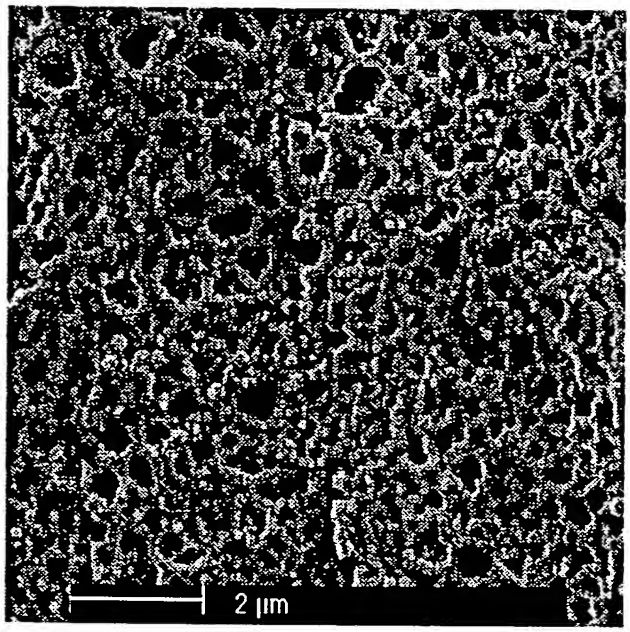
Figure 10



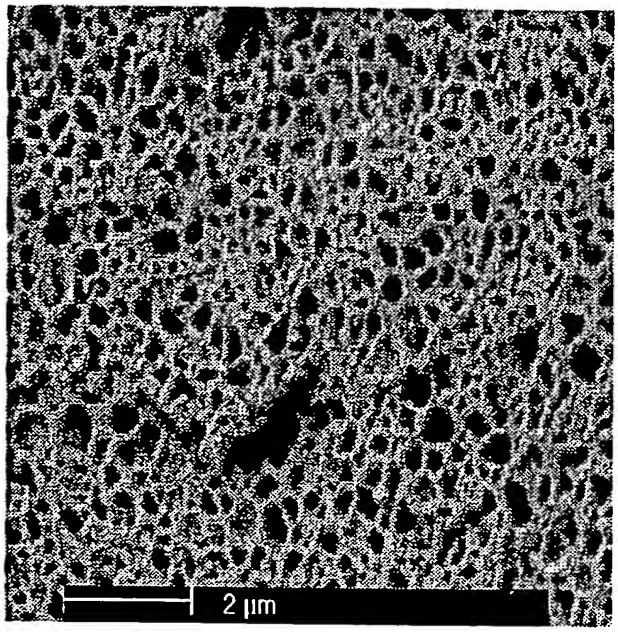
Bis



mBis



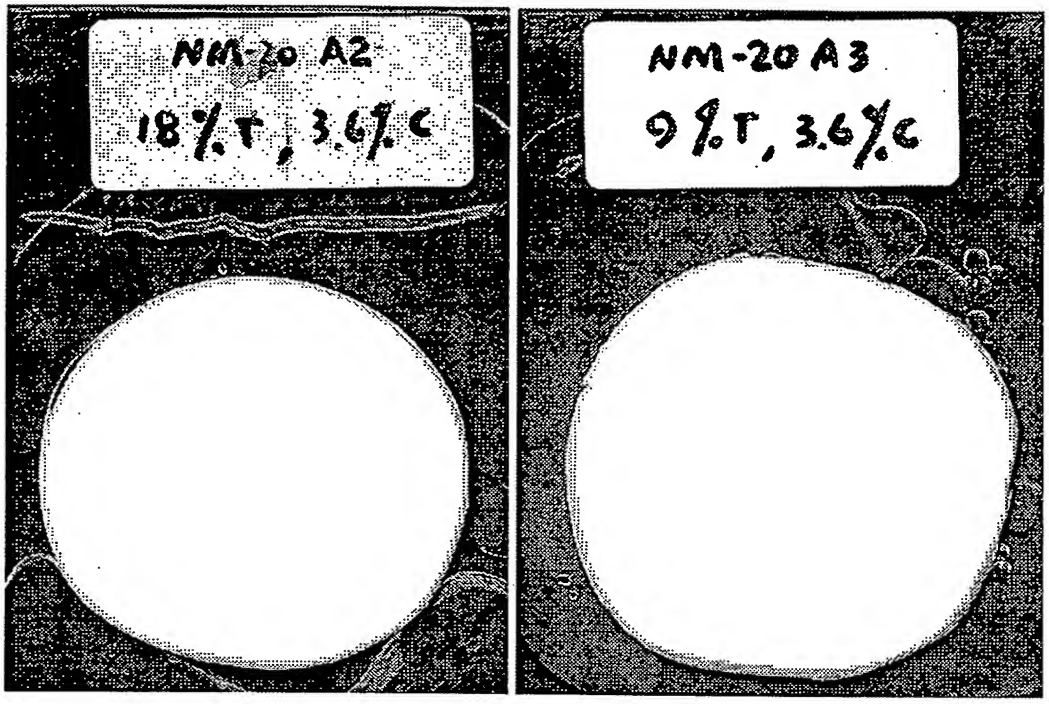
1a



1b

Figure 11

HEMA/EGDMA gels



HEA/EGDMA gels

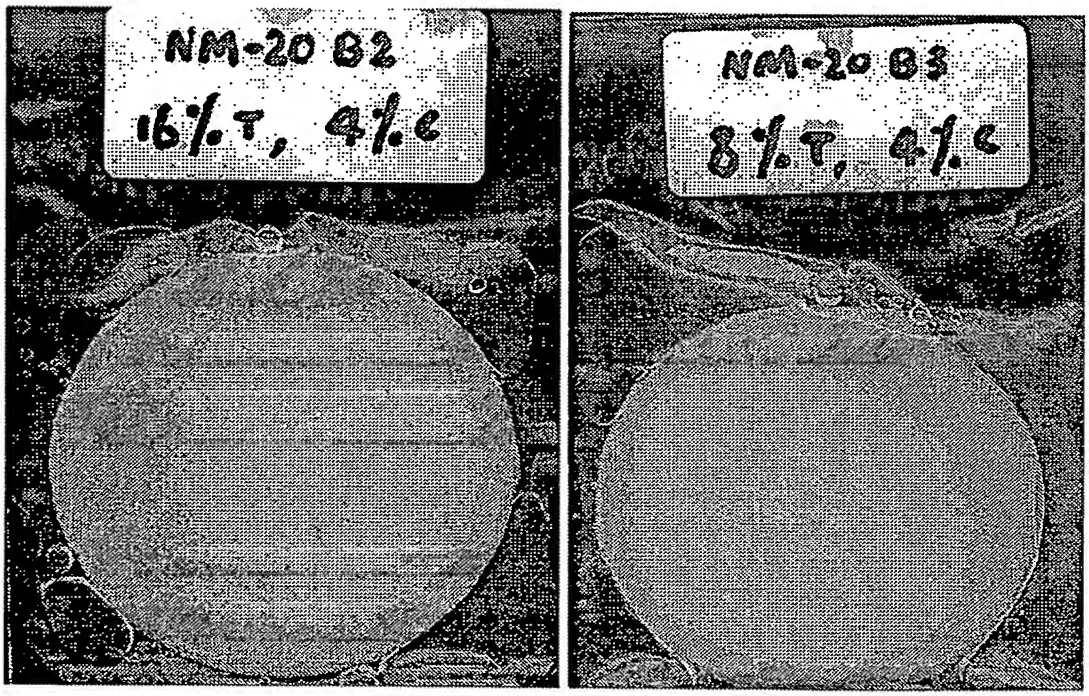


Figure 12